limitation "the order of the polynomial." This rejection was addressed by amending claim 6, upon which claim 8 depends, to depend from claim 2, which contains the proper antecedent basis. Similarly, claim 5 was amended to depend from claim 2 so that a sufficient antecedent basis is established for "the polynomial."

The Examiner's rejection of claims 9 and 10 is based on the recitation of multiple experimental traces in those claims, whereas these claims depend from claim 1 that only recites a singular experimental trace. This rejection was addressed by amending claim 1 to reflect the proper antecedent basis for claims 9 and 10 by reciting one or more experimental data traces instead of a singular experimental data trace. Claim 3 was amended similarly.

The Examiner's rejection of claim 14 is also based on an antecedent basis problem. This rejection was addressed by continuing to recite experimental data traces in the plural as contained in claim 11 upon which claim 14 depends. Similarly, claim 13 was amended to recite experimental data traces in the plural as contained in claim 11 upon which it depends.

In response to the Examiner's rejection of claim 15, Applicants respectfully traverse this rejection. In claim 15, the multiple separations can take place in a common lane, in a common gel, or across multiple gels. No amendment was made in response to the Examiner's rejection of this claim because Applicants contend that the multiple separations of the reference fragments can take place in all of the media and positions listed by the Examiner. Applicants respectfully submit that this claim is definite as written, and that they should not be required to list all possibilities within this set. Applicants respectfully request withdrawal of this rejection.

The Examiner's rejections to claims 16 and 19 were based on insufficient antecedent basis for the limitation "the order of the polynomial" or "the polynomial." This rejection was addressed by amending claim 16 to depend from claim 12, which contains the proper antecedent basis. Similarly, claim 17 was amended to depend from claim 12 so that a sufficient antecedent basis is established for claim 19, which depends from claim 17.

35 USC §102 Rejections

Koutny, et al. The Examiner rejected Claims 1, 2, 5-13, and 16-22 as being anticipated by Koutny, et al. Applicants respectfully traverse this rejection. The Examiner has

characterized claim 1 of the present invention in a manner that is substantially erroneous. For example, the Examiner states that the reference trace "has been corrected in its time scale to make the spacing of peaks fairly regular." This however, is not what is done, or what is claimed. Rather, a corrected time scale is developed for the reference trace, so that the peaks appear at the right places within a framework of regular spacing. This is possible because it is known where in the trace peaks should occur (because the reference sequence is known); one can assign observed times to known fragment lengths.

The Examiner continues by saying that the next step is assignment of base numbers to peaks which appear in the same place (time) as peaks in the reference. This presupposes that the reference is the same as the experimental, and does not allow for assignment of base numbers to non-corresponding peaks. In fact, the claim calls for sampling of the experimental trace at the times indicated by the time scale and assigning peak numbers only to those peaks which occur at these times (and thus not to peaks which may occur at other times). Thus, it does not appear that the Examiner has correctly understood the invention.

The Koutny reference does not teach the presently claimed invention. Koutny addresses correction of band distortion in a sequencing gel autoradiograph.. An image is acquired of the processes gel, and this image is analyzed on a pixel-by-pixel basis and adjusted in a two step process. The first step is referred to as "intralane straightening." Peaks are identified within a gel lane. Then, the first pixel column is selected as a "standard" for the band and a polynomial is identified which makes the other pixel columns for the same band line up best. All this does is make the peak bands into straight instead of bent bands. This does not correct the time scale as the Examiner suggests. The second phase of the process is "interlane straightening." This is not very clearly described, but appears to involve using the polynomial determined for one lane as a starting point to determine a starting polynomial for an adjacent lane. No reference trace is used to define a corrected time scale, and no sampling of the experimental trace takes place based on such a corrected time scale. Furthermore, we note that Figure 7 which the Examiner characterizes as showing normalization in fact only shows that similarity of band spacing was achieved using the Koutny methodology.

Since the Koutny, et al. reference does not teach the presently claimed invention and the

Examiner's rejection is based on an erroneous interpretation of the presently claimed invention, Applicants respectfully request withdrawal of this rejection and allowance of Claims 1, 2, 5-13, and 16-22 as amended.

Gabe, et al. The Examiner rejected Claims 1, 3, 6, 7, 11, 13, 14, 17, 18 and 21 as being anticipated by Gabe, et al. (US 5,981,186) Applicants respectfully submit that Gabe, et al. does not teach the presently claimed invention. The Examiner states that Gabe, et al. disclose methods of assigning base numbers to peaks of DNA sequencing data traces and that the sequencing reactions can be run in a single lane if the labels for each reaction are distinguishable, or they can be run in differing lanes on a common gel. The Examiner further states that Figure 1 is an example of base numbers assigned to two traces, and that the different traces can be aligned and normalized to have a standard spacing between the peaks - a method of correcting a time scale. The Examiner further states that an apparatus for performing the methods, which comprise a processor, input, output, and particular algorithms was disclosed in the Gabe reference. The Examiner appears to have taken the position that any method of correcting a time scale anticipates the claims. There are a number of possible methods of correcting a time scale. The claims of the present invention are directed to a particular method, and this method is not disclosed by Gabe, et al.

In the present invention an experimental data trace representing the positions of a first species of base within a target polynucleotide and a reference data trace representing the positions of a second species of base (which may be the same as or different from the first species) within a reference polynucleotide are obtained by separating appropriate sequencing fragments generated from the target and reference polynucleotides in a common lane of an electrophoresis gel. For each reference data trace, a plurality of peaks corresponding to fragments having a size in the range of 40 to 1200 bases are selected. A base number is assigned to each of the selected peaks in the reference data trace, and a numerical "peak file" is created with information about the peak number and migration time (or distance). This peak file is analyzed to determine a set of polynomial coefficients which will allow substantial linearization of a plot of peak number versus separation between adjacent peaks and alignment of the traces with respect to each other. These coefficients are used to create a corrected time scale identifying

where peaks should be located on a given experimental gel. This corrected time scale is used to guide the sampling of the experimental data, and for assignment of peaks within the data. The process of linearization and alignment is essentially one of assigning a correct numerical position to each of the bases. An important aspect of the linearization and alignment process is compensation for variation in peak spacing which occurs over time even within a single lane of an electrophoresis gel. The present invention performs this compensation by co-electrophoresing a reference sequence with the experimental sequence and utilizing the resulting reference data trace to define the correct peak spacing.

In Gabe, et al. a different method for correcting a time scale is taught. In Gabe, et al. two chain-extension sequencing reactions are performed on the target nucleic acid polymer to produce two reaction mixtures. The first reaction generates fragments terminated at two of the four standard nucleotides by having two types of chain terminators present in the reaction. The second reaction generates fragments terminating at two of the four standard nucleotides using two types of chain terminators, one of which is also used in the first reaction and the other of which is not. The reaction products of each reaction are separated on a length basis, generally by electrophoresis, detected and reported in a chromatogram format containing two data channels, one for each reaction mixture. Data analysis is based on a comparison of peaks present in the two data channels. Peak assignment is based on the type of chain terminators present in the reaction mixtures. Additional data treatment is applied to compensate for "real world variability," such as variability in peak height and spacing.

Since Gabe, et al. do not teach the presently claimed invention, Applicants respectfully request withdrawal of this rejection and allowance of Claims 1, 3, 6, 7, 11, 13, 14, 17, 18 and 21 as amended.

Green, et al. The Examiner rejected Claims 1, 2, 4, 6-13, 15, and 17-22 as being anticipated by Green, et al. (US 5,853,979) Applicants respectfully submit that Green, et al. does not teach the presently claimed invention. The Examiner states that Green, et al. disclose methods for analysis of DNA sequence traces wherein experimental data is compared to a reference trace. In the Green patent, the standard data trace is one which should (absent mutation or error) be the same as the experimental fragment. The analysis of the two traces is done to

determine the stretching and shifting which is needed to make the experimental trace look like the standard. There is no analysis of the standard data trace to find the times at which peaks should occur, and then use of this time scale to sample for peaks.

Since Green, et al. do not teach the presently claimed invention, Applicants respectfully request withdrawal of this rejection and allowance of Claims 1, 2, 4, 6-13, 15, and 17-22 as amended.

Gilchrist, et al. The Examiner rejected Claims 1, 2, 4-13, and 15-22 as being anticipated by Gilchrist, et al. (US 5,916,747). Applicants respectfully submit that Gilchrist, et al. does not teach the presently claimed invention. The Examiner states that the Gilchrist patent discloses methods for analysis of DNA sequence traces wherein one or more experimental data traces are compared to a reference trace. Applicants respectfully submit that the point in this patent is the use of the four traces to provide the alignment information; no reference is used. The Gilchrist patent discloses a method for aligning data traces from four channels of an automated electrophoresis detection apparatus in which each channel detects the products of one of four chain-termination DNA sequencing reactions such that the four channels together provide information concerning the sequence of all four bases within a nucleic acid polymer being analyzed. The method places the four data traces in a trial alignment, and then determines coefficients of shift and stretch for selected data points within each normalized data trace to optimize a cost function which reflects the extent of overlap of peaks in the combined normalized data traces to which the coefficients have been applied. Warp functions are then generated for the normalized data traces from the coefficients of shift and stretch determined for the selected data points, and applied to the respective data trace to produce four warped data traces which are assembled to form an aligned data set. This data set is then used for base-calling to complete the sequence determination process.

Since Gilchrist, et al. do not teach the presently claimed invention, Applicants respectfully request withdrawal of this rejection and allowance of Claims 1, 2, 4-13, and 15-22 as amended.

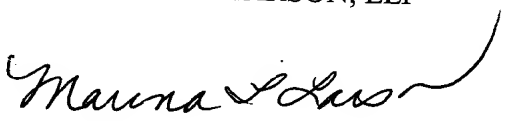
Imai, et al. The Examiner rejected Claims 1, 11 and 21 as being anticipated by Imai, et al. (US 5,891,632). Applicants respectfully submit that Imai, et al. does not teach the presently

claimed invention.

This patent contains no teaching relevant to the claimed invention. For example, Figure 2 (on which the Examiner has pointed to the labeled base numbers) is simply an example of how sequencing data may be displayed. There is no teaching with respect to this figure concerning how the base numbers were assigned or how the data traces were processed and analyzed. Furthermore, the actual invention of the Imai patent is a display for allowing visual matching of traces, for example when fragments are being assembled into a completed sequence. There is no "reference trace" as that term is used in the current application. Furthermore, the alignment which is done is done after assignment of base sequences. Thus, the basis for this rejection is unclear. Since Imai, et al. do not teach the presently claimed invention, Applicants respectfully request withdrawal of this rejection and allowance of Claims 1, 11 and 21 as amended.

For the foregoing reasons, Applicants respectfully urge that all of the claims and drawings as now presented be allowed.

Respectfully Submitted,
OPPEDAHL & LARSON, LLP



Marina T. Larson, Ph.D.
Reg. No. 32,038
P.O. Box 5068
Dillon, CO 80435-5068
970-468-6600

MARKED UP VERSION OF AMENDED SPECIFICATION

The last paragraph on page 9 beginning at line 22 and extending through line 4 on page 10:

Fig. 6 shows a schematic representation of an apparatus in accordance with the present invention for evaluating the sequence of a target polynucleotide. The apparatus as shown comprises a processor housing 10 which has an input 11 for receiving information from a sequencer 12 about one or more experimental DNA sequencing data traces derived from the separation of experimental DNA sequencing fragments reflecting the position of at least one base in the target polynucleotide and one or more reference DNA sequencing data traces derived from the separation of reference DNA sequencing fragments reflecting the position of at least one base in a reference polynucleotide of known sequence. For example, input 11 may be in the form of a wire for transmitting sequence-related data from a sequencer. Data could also be transmitted via a wireless link, or communicated to the apparatus through disk drive 13.

The last paragraph on page 12 beginning at line 23 and extending through line 2 on page 13:

Figs. 1 and 2 illustrate the application of the invention to the specific sequences described above. An M13 sequence using T-terminated sequencing fragments was performed with T = 6% and run at 60°C on a long gel with a voltage of 1500v. Fig[,]. 1 shows the spacing between adjacent bases as a function of base number, for non-aligned (raw) data (closed diamonds), and data aligned and linearized using a 3rd order (open triangles) and 5th order (open circles) polynomials.[.] It is clearly seen that the spacing is changing during the run significantly, but is linearized by fitting with either the 3rd or 5th order polynomial.

MARKED UP VERSION OF AMENDED CLAIMS

(Amended) A method for assignment of base numbers to peaks within [an] one or more experimental DNA sequencing data traces derived from the separation of experimental DNA sequencing fragments, comprising the steps of:

(a) obtaining one or more reference DNA sequencing data traces derived from the separation of reference DNA sequencing fragments reflecting the position of at least one base in a reference polynucleotide of known sequence;

(b) evaluating the reference DNA sequencing data traces to determine a corrected time scale indicative of migration times at which peaks should occur;

(c) sampling the experimental DNA sequencing data trace(s) at time points determined by the corrected time scale, and

(d) assigning a base number to each peak found in the experimental DNA sequencing data trace(s) based upon the corrected time scale.

3. (Amended) The method of claim 1, wherein the experimental DNA sequencing data traces and a first reference DNA sequencing data trace are derived from analysis of sequencing fragments in a common lane of a sequencing gel.

5. (Amended) The method of claim [1] 2, wherein the polynomial is a third or higher order polynomial.

6. (Amended) The method of claim [1] 2, wherein a defined number of bands are selected for evaluation from each of the reference DNA sequencing data traces.

13. (Amended) The method of claim 11, wherein the reference DNA sequencing traces and the experimental DNA sequencing data traces are derived from analysis of sequencing fragments in a common sequencing gel.

14. (Amended) The method of claim 13, wherein the experimental DNA sequencing data traces and a first reference DNA sequencing data trace are derived from analysis of sequencing fragments in a common lane of the common sequencing gel.

16. (Amended) The method of claim [11] 12, wherein the polynomial is a third or higher order polynomial.

17. (Amended) The method of claim [11] 12, wherein a defined number of bands are selected for evaluation from each of the reference DNA sequencing data traces.